Inhibition of Hemangioendothelioma *in Vivo* and Invasion and Growth *in Vitro* by a Unique Nutrient Mixture

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Hemangioendothelioma

Introduction

Hemangiomas, the most frequent vascular tumors in Caucasian infants, occur in approximately 1% of normal newborns, but the incidence increases to 20% in premature infants weighing less than 1000 grams.

These lesions are characterized by rapid proliferation of capillaries during the first year of life, followed by a slowed growth and regression of the tumor over the next 5-6 years, with complete regression of the lesion by the age of 6-12 years.

Hemangioendothelioma

Introduction

- Approximately 5% of hemangiomas cause serious tissue damage, while 1-2% are life threatening.
- The pathogenesis of these tumors is still largely unknown and the current therapy, such as systemic corticosteroid, vincristine, and interferon-alpha, is toxic and remains unsatisfactory.
- A nutrient mixture (NM) containing lysine, proline, ascorbic acid, and green tea extract has shown significant anti-angiogenic and anti-tumor effects against a number of cancer cell lines.

Composition of Nutrient Mixture

Nutrient	Molar Concentration (in 100 µg/ml solution)
Vitamin C	90 µM
L-Lysine	110 µM
L-Proline	110 µM
L-Arginine	50 µM
N-Acetyl Cysteine	25 µM
Green Tea Extract	EGCG 15 µM
Selenium	8.5 µM
Copper	700 nM
Manganese	400 nM

In Vivo Study - Method

Using a mouse hemangioendothelioma model, we investigated the efficacy of NM.

- **Athymic nude mice, 5-6 weeks of age, were inoculated with $3x10^6$ EOMA cells (ATCC) subcutaneously and randomly divided into two groups; group A was fed a regular diet and group B a regular diet supplemented with 0.5% NM.**

- **Four weeks later, the mice were sacrificed and their tumors were excised, weighed, and processed for histology**

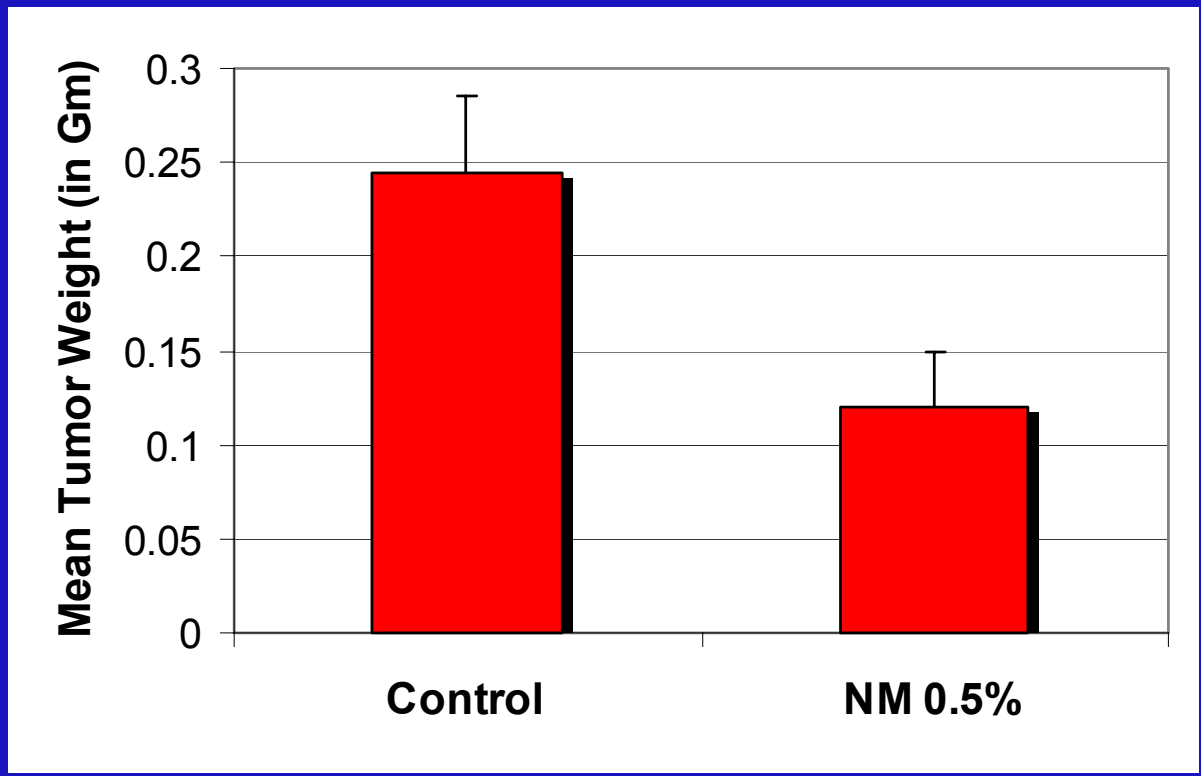
In Vitro Study - Method

We also tested the effect of NM *in vitro*, evaluating its effect on EOMA cell viability, MMP secretion, invasion, morphology, and apoptosis.

- Cell proliferation was measured by MTT assay
- Invasion through Matrigel
- Morphology by H&E staining
- Secretion of MMPs by gelatinase zymography
- Apoptosis by image-IT live green poly caspases detection kit

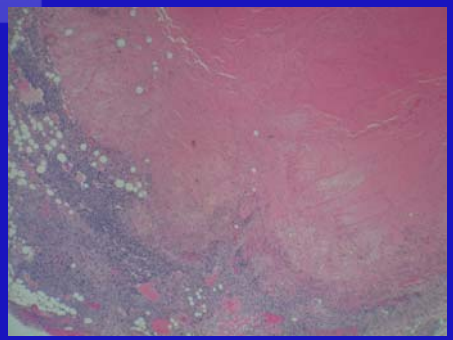
In Vivo Study
Tumor Growth

The nutrient mixture (NM) inhibited the growth of tumors by 50% (p=0.0001).

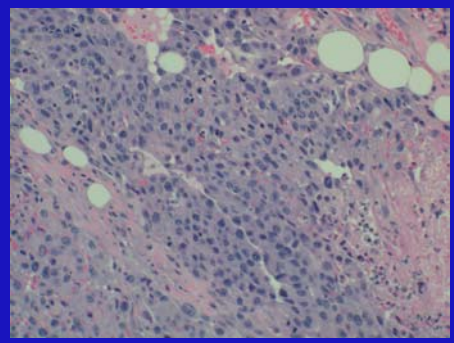


In Vivo Study
Tumor Histopathology

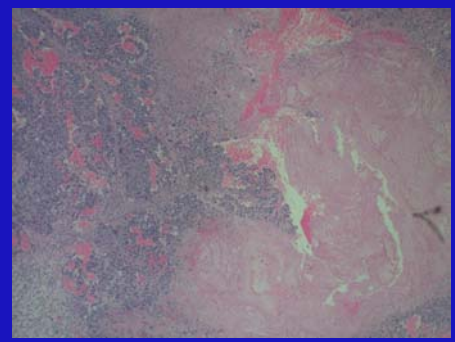
The tumors from both control and supplemented mice were round, highly vascular, invasive tumors consistent with cavernous hemangiosarcoma.



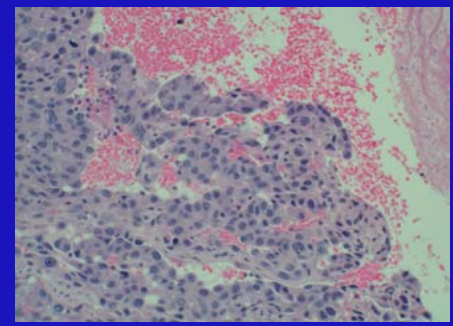
Control 40 x



Control 200x



NM 0.5% 40x



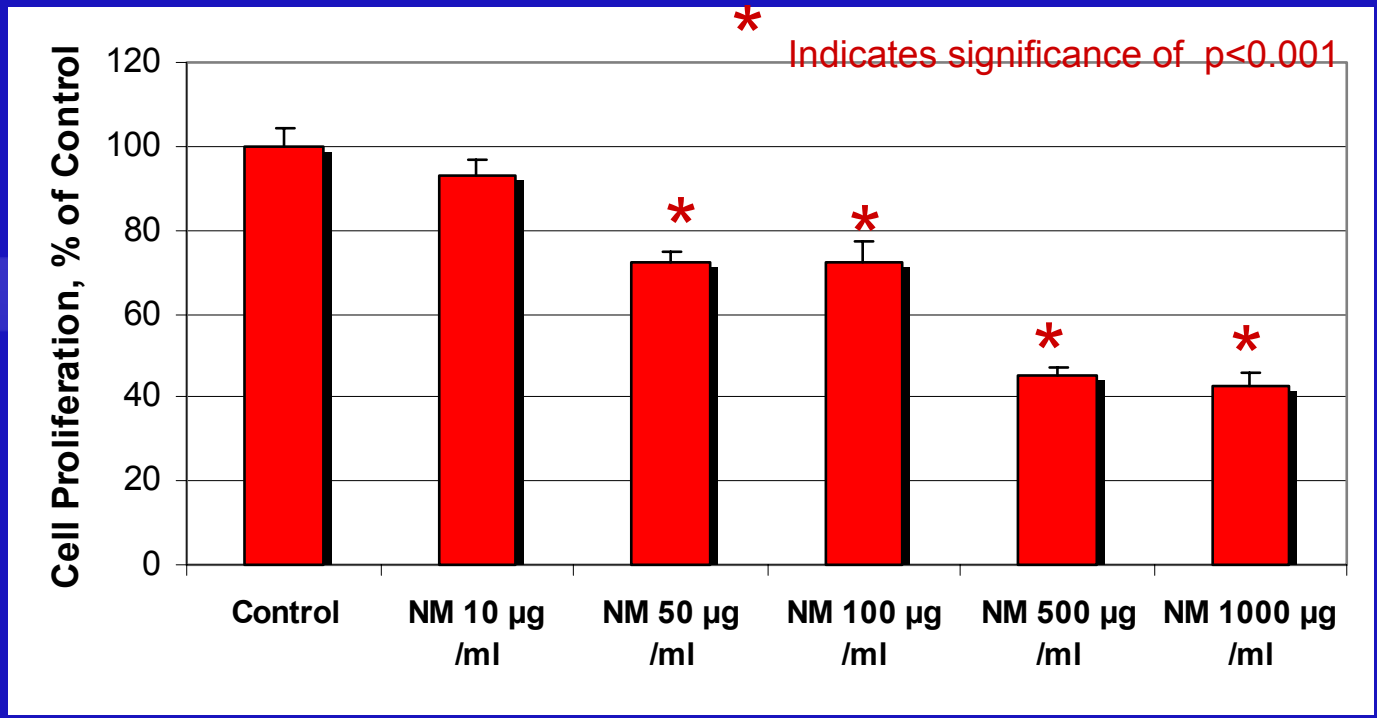
NM 0.4% 200x

Control group specimen – characterized as cavernous hemangiosarcoma with extensive thrombosis and necrosis.

Supplemented (NM 0.5%) group specimen – characterized as cavernous hemangiosarcoma with area of thrombosis .

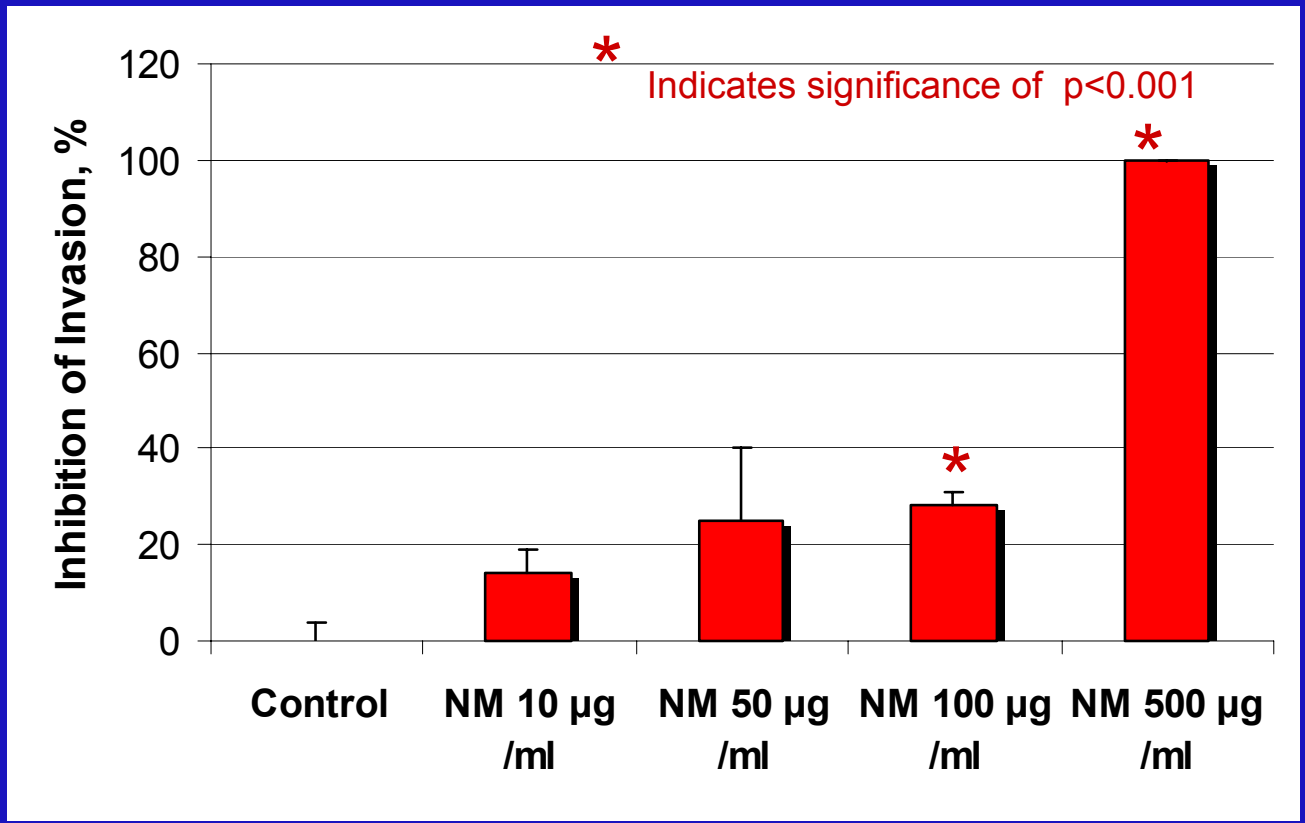
In Vitro Studies
MTT Assay – 24h

The MTT assay demonstrated dose-dependent toxicity with increased NM concentration – 10%, 30%, and 55% at 10, 100, and 1000 µg/ml respectively.

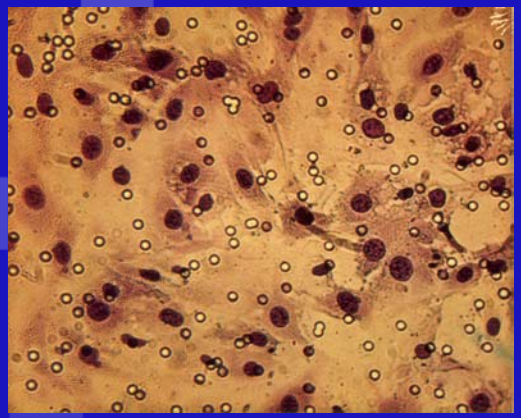


In Vitro Studies
Matrigel Invasion

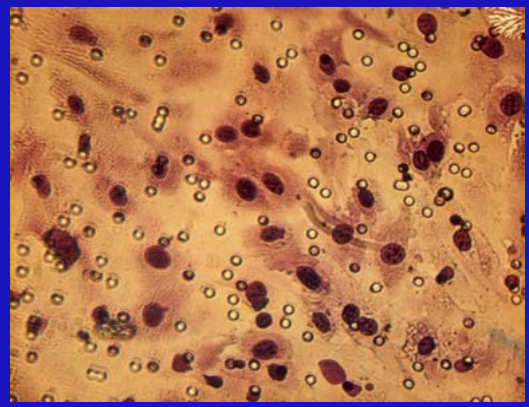
Invasion of EOMA cells through Matrigel was inhibited at 10, 50, 100, and 500 µg/ml by 14%, 25%, 28%, and 100% respectively



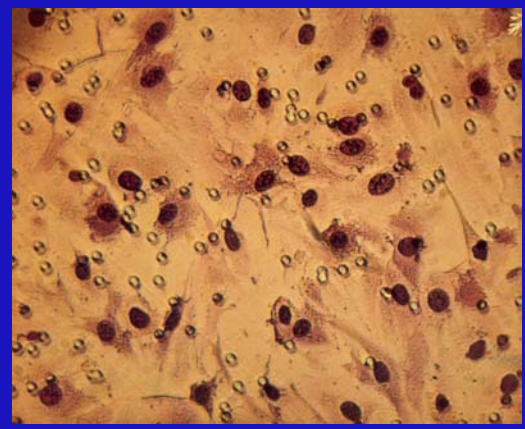
In Vitro Studies
Invasion photomicrographs



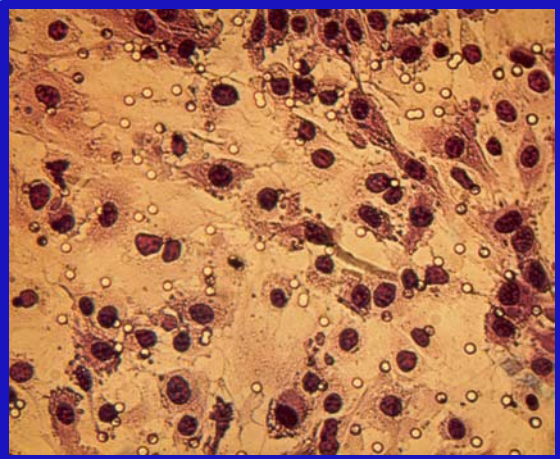
Control



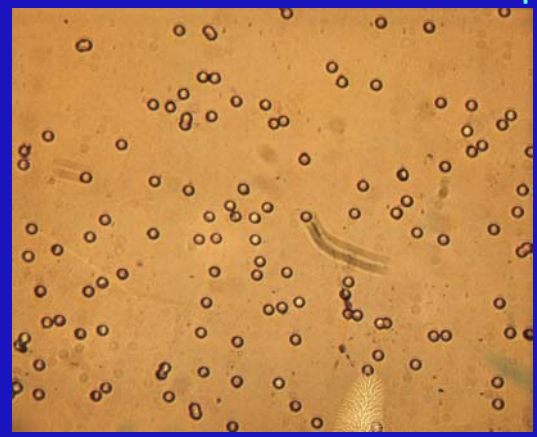
NM 10 µg/ml



NM 50 µg/ml



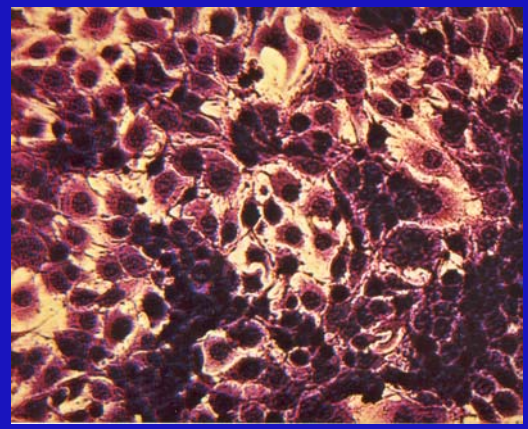
NM 100 µg/ml



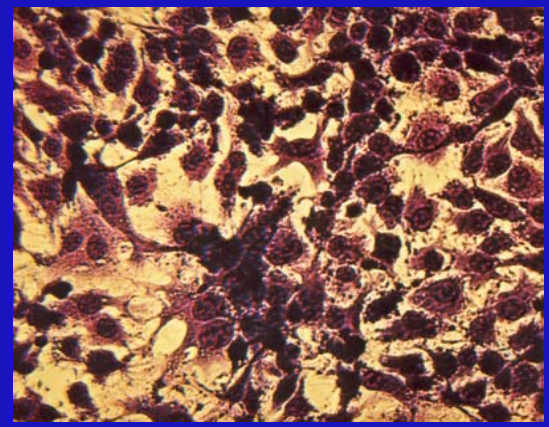
NM 500 µg/ml

In Vitro Studies
Morphology – H&E

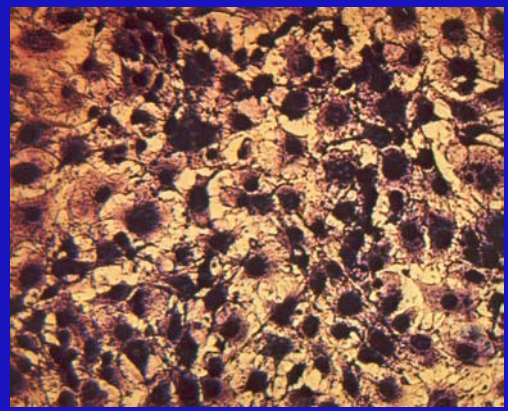
H&E did not indicate any EOMA cell morphology changes even at exposure to the highest NM concentration



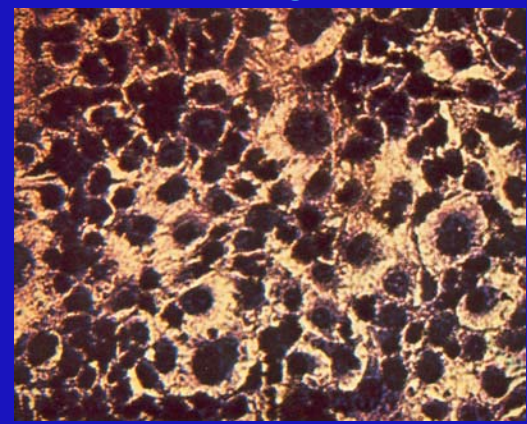
Control µg/ml



NM 50 µg/ml



NM 500 µg/ml

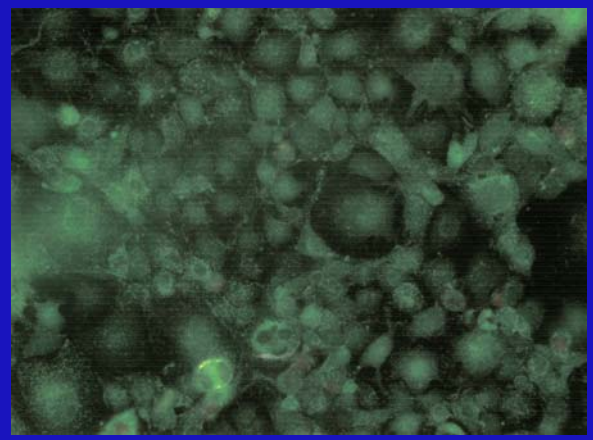


NM 1000 µg/ml

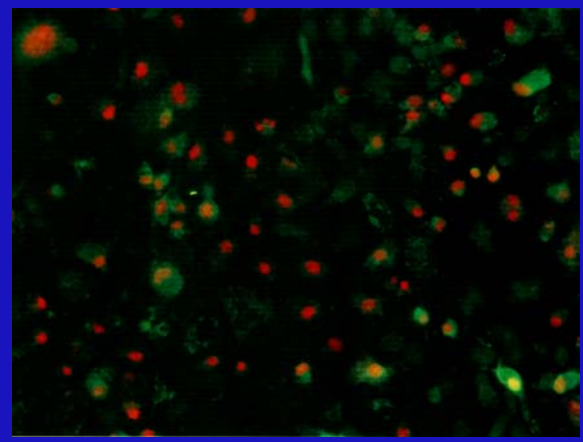
In Vitro Studies

Apoptosis (caspase activity)

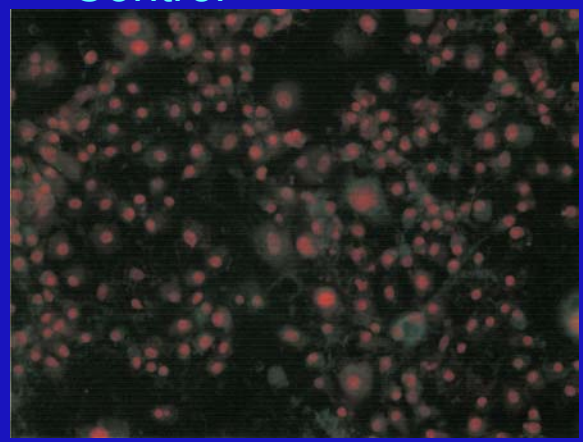
Exposure of EOMA cells to NM resulted in slight apoptosis (assessed by image-IT live green poly caspases detection kit) at 100 µg/ml, moderate at 500 µg/ml and potent at 1000 µg/ml.



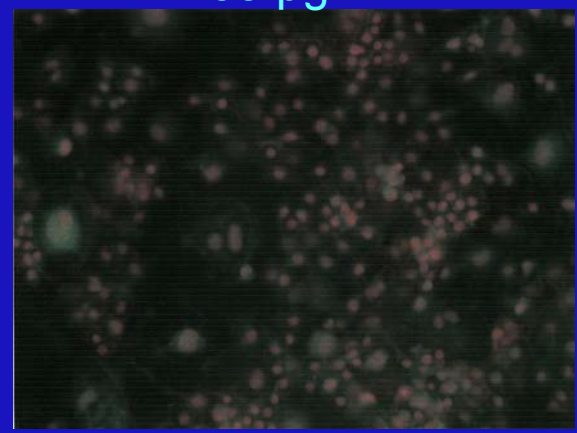
Control



NM 100 µg/ml



NM 500 µg/ml



NM 1000 µg/ml

Conclusions

Our results suggest that NM may have therapeutic potential in treating infantile hemangioendotheliomas and, perhaps, other cutaneous vascular tumors.

For further information on research activities, publications, and testimonials, visit our research website at:

http:// www.drrathresearch.org